Medical Science

pISSN 2321–7359; eISSN 2321–7367

Lactobacillus acidophilus' post biotic extracts combined with methotrexate regulated the levels of the apoptotic genes' expressions in the acute lymphoblastic leukemia cell line

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To Cite:
EL Hadad S, Baik H, Alhebshi AM, Aburas M, Alrahimi J, Hassoubah S. *Lactobacillus acidophilus'* post biotic extracts combined with methotrexate regulated the levels of the apoptotic genes' expressions in the acute lymphoblastic leukemia cell line. Medical Science, 2022, 26, ms111e2020. doi: https://doi.org/10.54905/disssi/v26i122/ms111e2020

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Peer-Review History
Received: 13 December 2021
Reviewed & Revised: 19/December/2021 to 19/March/2022
Accepted: 21 March 2022
Published: 01 April 2022

Peer-review Method
External peer-review was done through double-blind method.

URL: https://www.discoveryjournals.org/medicalscience

ABSTRACT

Acute lymphoblastic leukemia (ALL) cure efficacy depends upon the chemotherapy drugs' effectiveness, the leukemia cells' biological traits, and the early response to treatment. The apoptotic genes' pivotal roles on different stages of cancer growth and drug resistance were attracted most immunologists and chemotherapeutic developers. Lactobacillus (L) probiotic bacteria or postbiotics have antitumor impacts that enhance the apoptosis process. Hence, to examine the apoptotic effects of two different concentrations of *L. acidophilus* postbiotics and Methotrexate (MTX) chemotherapy on the ALL propagation; four groups of ALL cell line were treated as follow: N group; cells kept untreated (negative control), M group; cells were treated by 0.236 mg/ml of MTX (positive control), accompanying with two different ALL-cells groups treated as similar as M group in addition to either 0.5 ug/ml (ML5 group) or 2 ug/ml (ML20 group) of *L. acidophilus* postbiotics. We estimated the transcription levels of different apoptotic gene markers after twenty-four and forty-eight hours from treatments. We noted an earlier extremely significant elevation in the transcription levels of *BAX*, *NF-kB*, *Notch1*, and *Notch2* genes in the ML5 cells group compared to the ML20, N, and M cells groups. The transcription levels of the *JAG1* and *JAG2* genes diminished significantly in the ML5 cells compared to the ML20, N, and M cells groups. Consequently, these results verified that the *L. acidophilus* postbiotics have an early positive role on the transcriptions levels of apoptotic genes during treatment with the Methotrexate chemotherapy and may directly impact the apoptosis process considered the heroine of cancer elimination.

Keywords: Acute lymphocytic leukemia; Lactobacillus acidophilus; apoptosis genes; antitumor immune response; Probiotics.

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL) is an unpleasant alteration that occurred in the B and T cells ancestors (lymphoid progenitors) (Huntly and Gilliland, 2005). This single transformed hematopoietic precursor proliferates and losses the differentiation causing suppression of the normal lymphoid cells' maturation (Graux, 2011). Commonly, B and T ALL were the most established ALL types, where more than 80% of ALL are B-ALL, and about 20% are T-ALL (Graux, 2011). In Saudi Arabia, Childhood cancer represents 6.1% of all cancers, whereas the highest incidence of 31% is counted for ALL (Saudi Cancer Registry MoH, 2013). Generally, most cancer chemotherapy mechanisms depend upon encouraging the apoptosis process either by the intrinsic; or the extrinsic apoptosis pathways (Kim, 2005). ALL-patients may hurt from the most unfortunate complications among leukemia patients; wheresoever, most ALL treatments cause drug resistance, either single drugs or combinations of drugs (Den Boer *et al*., 2003).

Methotrexate (MTX) is an essential drug that is ordinarily applied as chemotherapy for several malignant and non-malignant illnesses (Conway and Carey, 2017). MTX is an analog and competitor of the folic acid cycle, thereby facilitating the apoptosis of the transforming cancerous cells (de Beaumais and Jacqz-Aigrain, 2012). Generally, the dose of the MTX differs according to the stage of the ALL disease, and their acuteness. It is given as either consolidation chemotherapy (high intravenous dose) or as maintenance therapy (low oral dose) (Csordas et al., 2014). MTX reduces the release of the IL8, TNF-α, and IL6 proinflammatory cytokines, the proliferation of the monocytes and macrophages, cellular immunity, and prevents the migration of the leukocytes to tissues (Wiewiórowski and Graczyk, 2000). Moreover, MTX hindered NF-κB signaling by activating the p53 in the T-ALL cells (Bedoui *et al*., 2019).

The immunomodulatory impacts of probiotic bacteria, particularly *Lactobacillus*, on human health attract the attention of scientists from several fields (Hill *et al*., 2014). Para-probiotics (non-viable microbial cells) and postbiotics (cell-free extracts) are new terms that implement a more obvious meaning to the impacts of the probiotic on human health (Cuevas-González *et al*., 2020). Many studies revealed mazing evidence that postbiotics and para-probiotics possess specific bioactive properties such as antimicrobial, antitumor, and immunomodulatory actions through direct or indirect pathways (Cuevas-González *et al*., 2020). *Lactobacillus* probiotic bacteria have high levels of microbial carbohydrates, including peptidoglycan and exopolysaccharides fractions and extracts; these have potent tumor suppressor effects (Choi et al., 2006, Ambalam et al., 2016). Moreover, direct exposure to *Lactobacillus* extracts and fractions inhibited some cancer cell lines through apoptosis process incitement (Karimi Ardestani *et al*., 2019).

Many previous studies verified the capability of some probiotics to hinder the growth of some cancers (Ding *et al*., 2018), especially colon cancer, but others probiotics have the contrary action (Hadad *et al*., 2021). Also, probiotics' antitumor activity boosts the efficacy of 5-FU chemotherapy activity against colon cancer through enhancing the mucosal immune responses (Hadad *et al*., 2019). It was previously unknown whether/whether not postbiotics of Lactobacillus can improve chemotherapeutic apoptosis efficiency, so the present study evaluated the anticancer combination activity of *L. acidophilus* bacteria and Methoteraxate in Acute lymphoblastic leukemia by investigating the apoptosis genes transcription levels.

2. MATERIALS AND METHODS

Human T- acute lymphoblastic leukemia culture

Human T- acute lymphoblastic leukemia (T-ALL cell line or Jurkat colone E6-1) was obtained from American Type Culture Collection (Rockville, MD, USA). The cells of T-ALL were cultivated in RPMI 1640 medium (UFC Biotech, Cat No-111241KSA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) from (Biosera, Cat No-FB-1001/500, South America) and 1% antibiotics (penicillin and streptomycin) (Gibco,15140122, USA). The T-ALL cells were cultured in 25 cm^2, then 75cm^2 tissue culture flasks (SPL, Korea). The incubation condition was 24 hrs at 37°C in a 5% CO2 incubator and the cells were regularly passaged till the viability of the cells reached 90%. Cells viability and counting were determined microscopically after staining with trypan blue (Strober, 2015).

***L. acidophilus* cultured, growth conditions, and cell-free supernatant preparation**

The specific strain of *L. acidophilus* was obtained from the yogurt sample product purchased from the local market in Jeddah, KSA. Before the *L. acidophilus* culturing, culture media de Man Rogosa Sharpe Agar medium (MRS) -usually utilized to enrich the *Lactobacillus* strains- were sterilized for 15 min at 121°C using an autoclave. The *L. acidophilus* bacteria were cultured in MRS at 37°C for 48 hrs in anaerobic conditions (Liu *et al*., 2020).

Regarding cell-free supernatant' preparation, the obtained *L. acidophilus* colonies were transferred in 50 ml of the MRS broth medium, actively sub-cultured twice, and incubated at 37C in a shaker incubator for two days. Then culture medium was centrifuged at 5000 x g for 20 min at 4°C for obtaining the culture supernatant. The *L. acidophilus* supernatants were lyophilized and stored at −20◦C until needed (Hansen *et al*., 2015).

Methotrexate chemotherapy

MTX-known as Amethopterin- is the familiar chemotherapeutic agent against childhood ALL disease (Hu *et al*., 2019). MTX tablets with a 2.5 ug/ml/tablet concentration were purchased from Ebewe Farma (Austria). Each MTX tablet was dissolved in 5 ml of RPMI-1640 medium to get a 0.236 mg/ml concentration. The dissolved MTX was filtered using a 0.22 μm filter disk then stored at −20◦C until needed.

Experiment design

This study was accomplished in King Fahed for Medical Research from November 2019 till April 2021 and the Ethical approval Reference No is 681-19, Tissue culture). The ALL-cells were randomly divided into negative untreated control (N group), positive control (M group), and two *L. acidophilus* treated groups (ML5 and ML20 groups). The ALL-cells from the N group were kept untreated and propagated in normal conditions. The ALL-cells related to the M group received one dose of a total of 0.236 mg/ml MTX drug over 48 hrs (from hr 0 to hr 48). The ALL-cells belonging to ML5 and ML20 groups were treated with one dose of 0.5 and 2 ug /mL of *L. acidophilus* cell-free supernatant, respectively, by the side of MTX drug treatment following the same protocol as that with the M group. Also, ML5 and ML20 groups were treated with *L. acidophilus* cell-free supernatant from 0 hrs till 48 hrs of the experiment. Next, three flasks of ALL cells from each group (N, M, ML5, and ML20) were harvested after 6, 24, and 48 hrs. The obtained ALL-cells samples were collected and stored at -80 °C until subjected to genes expressions studies.

Table 1 Sets of specific primers were used for genes expression quantitation using SYBR green qRT-PCR

Gene	*Polarity*	*Primer sequence (5'---'3)*	*Primer length*	*Nucleotide positions*	*Accession GenBank No*
NOTCH-1	*F*	GAC ATC ACG GAT CAT ATG GA	*20*	*960-985*	*XM_006498795*
	R	CTC GCA TTG ACC ATT CAA AC	*20*	*1462-1458*	
NOTCH-2	*F*	GAT GCC ACC TGA ACA ACT GC	*20*	*1356-1374*	*XM_017321385.*
	R	TGA CAA CAG CAA CAG CAA GG	*20*	*1649-1631*	
JAG1	*F*	AGC GAC CTG TGT GGA TGA G	*19*	*323-345*	*NM_011905.3*
	R	GGC TGG AGA CTG GAA GAC C	*19*	*387-366*	
JAG2	*F*	TCT CTG TGA GGT GGA TGT CG	*20*	*824-844*	*NM_001276445*
	R	CAG TCG TCA ATG TTC TCA TGG	*21*	*933-914*	
BAX	*F*	CCT GTG CAC CAA GGT GCC GGA ACT	*24*	*205-228*	*NR_027491.1*
	R	CCA CCC TGG TCT TGG ATC CAG CCC	*24*	*311-289*	
NF-kB	*F*	ATC CCA TCT TTG ACA ATC GTG C	*22*	*674-697*	*X_60470.1*
	R	CTG GTC CCG TGA AAT ACA CCT C	*22*	*887-865*	
GAPDH	*F*	GCA CCG TCA AGG CTG AGA AC	*20*	*260-290*	*AH001969.2*
	R	TGG TGA AGA CGC CAG TGG A	*19*	*967-937*	

Relative ratio quantitation of the apoptotic genes expressed on the ALL-cells

Preservation of the current ALL-RNA samples groups was applied by following the protocol instructions. Also, the RNeasy Midi kit (QIAGEN, Cat No. 75142) was applied for mRNA extractions from the several treated and untreated ALL-cells groups according to the producer's standard protocol. By using the current different RNA samples and the sets of specific primers (Table 1), the quantitation of various target genes expressed in the ALL-cells were determined using VERSO SYBR Green One step qRT-PCR ROX

Kit (Thermo Scientific Cat No. A-4105/A) (El Hadad et al., 2019; Hadad et al., 2021). The equation of 2-ΔΔCt was used for the relative transcription levels of the target genes calculations. GAPDH gene was used as a housekeeping gene (Végran *et al*., 2011).

Statistical methods

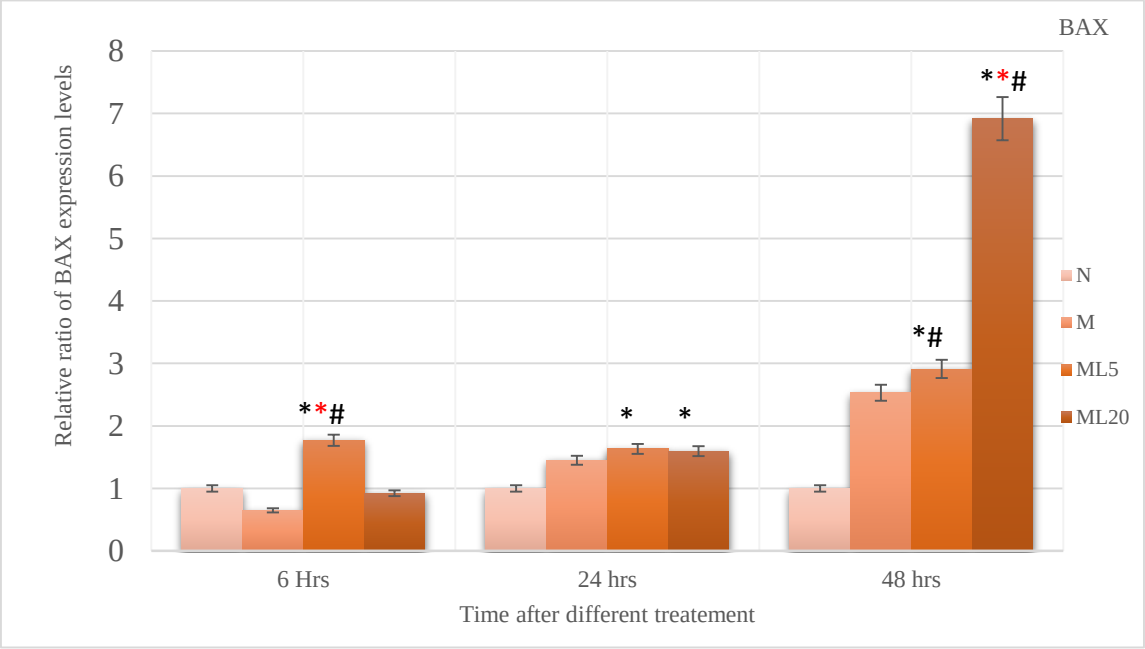
The statistical evaluations of the targeted genes expressions levels in the current T-ALL groups were performed using Megastat software version 10.1. The P-value < 0.05 was deemed significant.

3. RESULTS

Effects of the *L. acidophilus* free cell supernatant on the proapoptotic gene transcriptions

At hour six from treatment, *BAX* transcription level upregulated significantly in cells belonging to the ML5 group than those belonging to the untreated control, M, and ML20 groups (P= 0.0109, 0.0007, and 0.0059. respectively). Meanwhile, its level fluctuated nonsignificantly in the ML20 group than those belonging to the untreated and M cells groups in the same period (Figure 1). Sequential analysis of this pro-apoptotic gene transcription level after 24 hours showed an extremely significant increase in ML5 and ML20 cells groups compared to those detected in the untreated group (P= 0.0000 for each group); nevertheless, no significant differences in its expression level compared to its corresponding level either in the M group or in comparison with each other (Figure 1). Finally, at 48 hours, the *BAX* transcription level was significantly upregulated in the ML20 groups compared with its transcription levels in the untreated control, M, and ML5 groups (P-value= 0.0001, 0.0005, and 0.0050, respectively). No conspicuous discrepancies between the expression levels of this target gene in the ML5, M, and untreated groups were noted at hour 48 (Figure 1).

Concerning the *NF-κB* transcription level after six hours from treatment, no noticeable discrepancies were noted in the untreated control, M, ML5, and ML20 groups (Figure 1). By hours 24 and 48 from treatment, the transcription level of the *NF-κB* upregulated significantly in the ML20 group when compared with its level in cells belonging to the untreated control, M, and ML5 groups (P=0.0007, 0.0012, and 0.0011, respectively, for 24 hours) and (P= 0.0184, 0.0114, and 0.0234, respectively, for 48 hours). Its transcription level in the ML5 seemed more similar in comparison with those of the untreated control and M groups (Figure 1).



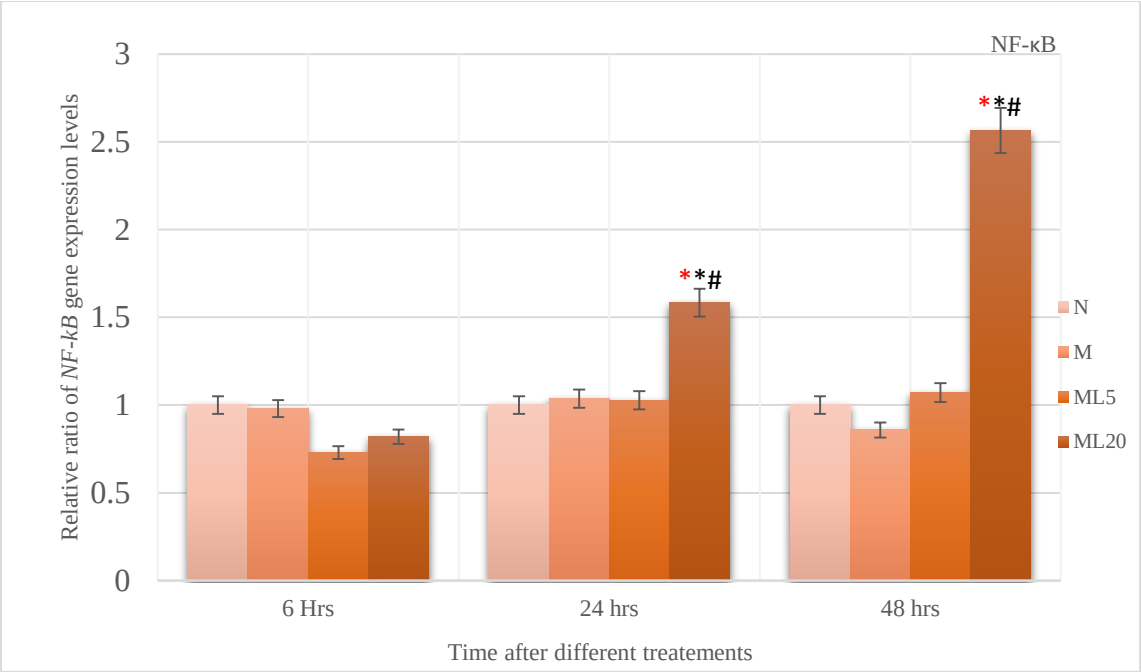
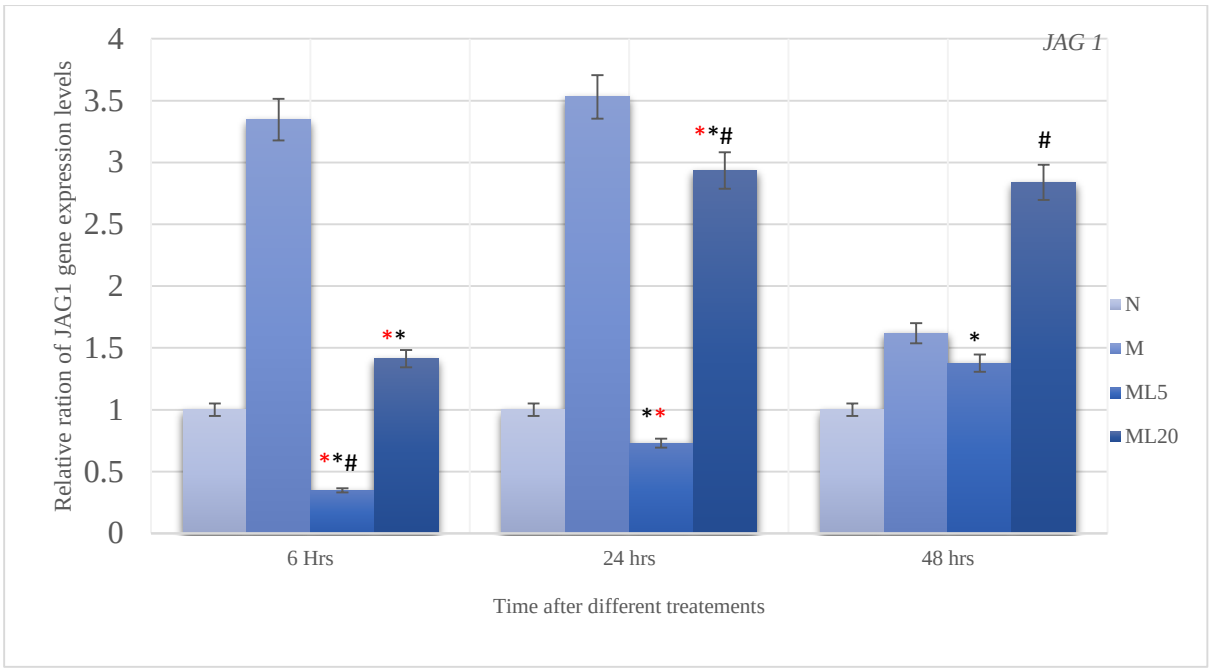


Figure 1 *BAX-mRNA and NF-κB-mRNA* profiles transcriptions level sin different treated and untreated ALL-cell lines' groups. *Where N Group represents untreated ALL-cell line as the negative control, M group represented ALL-cells treated with Methotrexate drug. ML5 and ML20 groups represent ALL cells treated with Methotrexate drug and 5 ug and 20 ug of Lactobacillus supernatant. A comparison was performed using the One-factor ANOVA test to analyse variance. (*) Significant at P<0.05. (*) Comparison between untreated control and treated groups. (#)Comparison between the M and the two concentrations of postbiotics probiotic treated groups. (*) Comparison between the three probiotic treated groups.*

Effects of the *L. acidophilus* free cell supernatant on the anti-apoptotic gene transcriptions

Changes in the *JAGGED1* (*JAG1*) and *JAGGED2* (*JAG2*) transcriptions levels were predestined at hrs 6, 24, and 48 from starting treatment. After six hours, the *JAG1* transcription level decreased significantly in the ML5 group compared to its level in either the untreated control, M, and ML20 groups (P= 0.0190, 0.000, and 0.0000, respectively). Meanwhile, cells belonging to the ML20 group showed an early non-significant upregulation in the *JAG1* expression than those belonging to the untreated group, however, but it inhibited significantly in comparison with its level in those belonging to the M group (P = 0.0000, Figure 2). By hour 24, the *JAG1* transcription level down regulated non-significantly in the ML5 group than the untreated group, but it was still significantly declined compared with its levels in those of the M and ML20 groups (P= 0.0000; for each group; Figure 2). Also, this anti-apoptotic gene transcription level elevated significantly in the ML20 cells compared to its corresponding level of the untreated cells group (P= 0.0000), but it significantly decreased compared to the M group (P = 0.0090; Figure 2). Finally, by hour 48, the *JAG1* transcription level swings non-significantly between all the treated and untreated groups including the ML5, ML20, untreated control, and M groups, despite its level increased significantly in the ML20 group compared to the untreated cells group (P= 0.0097; Figure 2).

About the *JAG2* transcription level after six hours from starting the treatment, it downregulated significantly in the ML5 group when compared to its corresponding levels in the M and ML20 groups (P = 0.0000/each group), though its level declined non-significantly compared to the untreated control group (Figure 2). Moreover, the *JAG2* transcription level was downregulated significantly in the ML20 group compared with its corresponding level of the M group (P= 0.0004). Its level increased significantly than those observed in the untreated cells group (P= 0.005) after the same period (Figure 2). After 24 hours, the transcription level of the *JAG2* gene showed a significant elevation in the ML5 cells than those belonging to the untreated cells (P= 0.0357). However, it's elevated non significantly compared to the M cells group. Also, the ML20 cells demonstrated an extremely significant upregulation in the transcription level of the *JAG2* gene compared to those belonging to the untreated control, M, and ML5 groups (P=0.0000/each group). Finally, by hour 48, the *JAG2* transcription level elevated significantly in the ML5 group than its corresponding level in the untreated, M, and ML20 groups. Also, no conspicuous differences in the expression levels of the ML20, untreated control, and M groups were noticed (Figure 2).



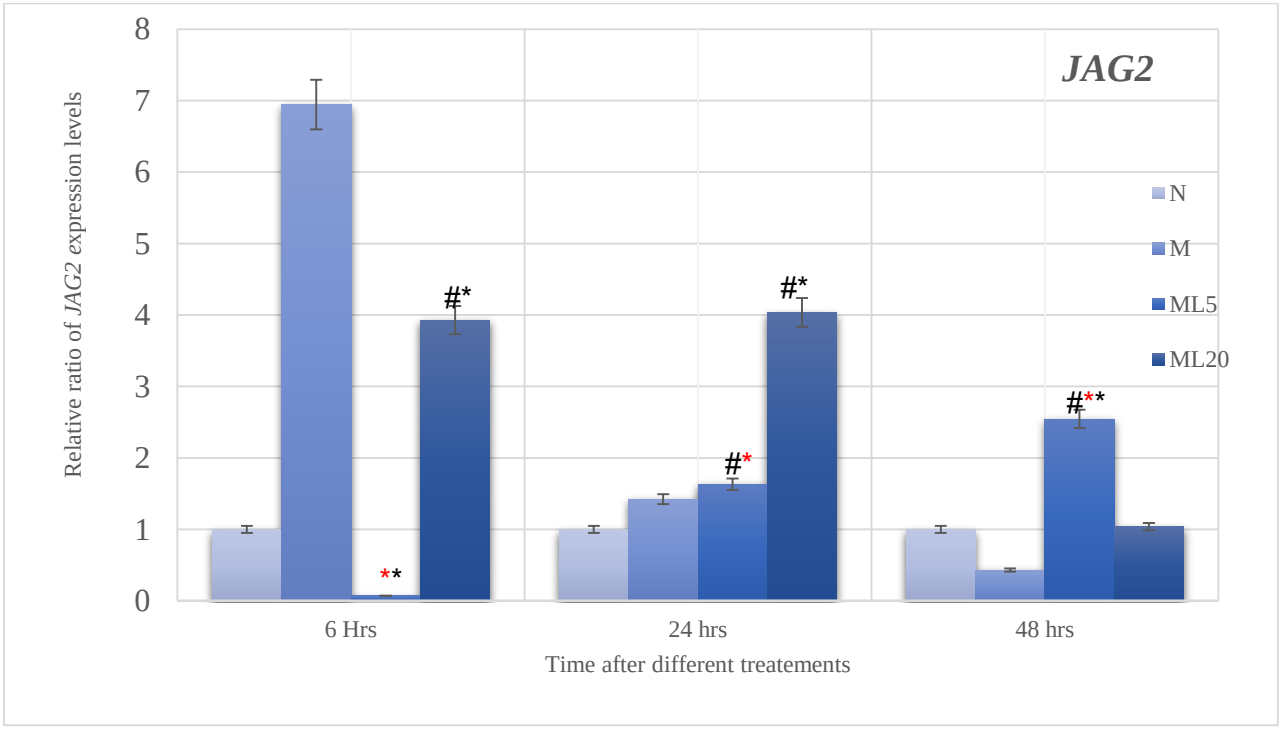


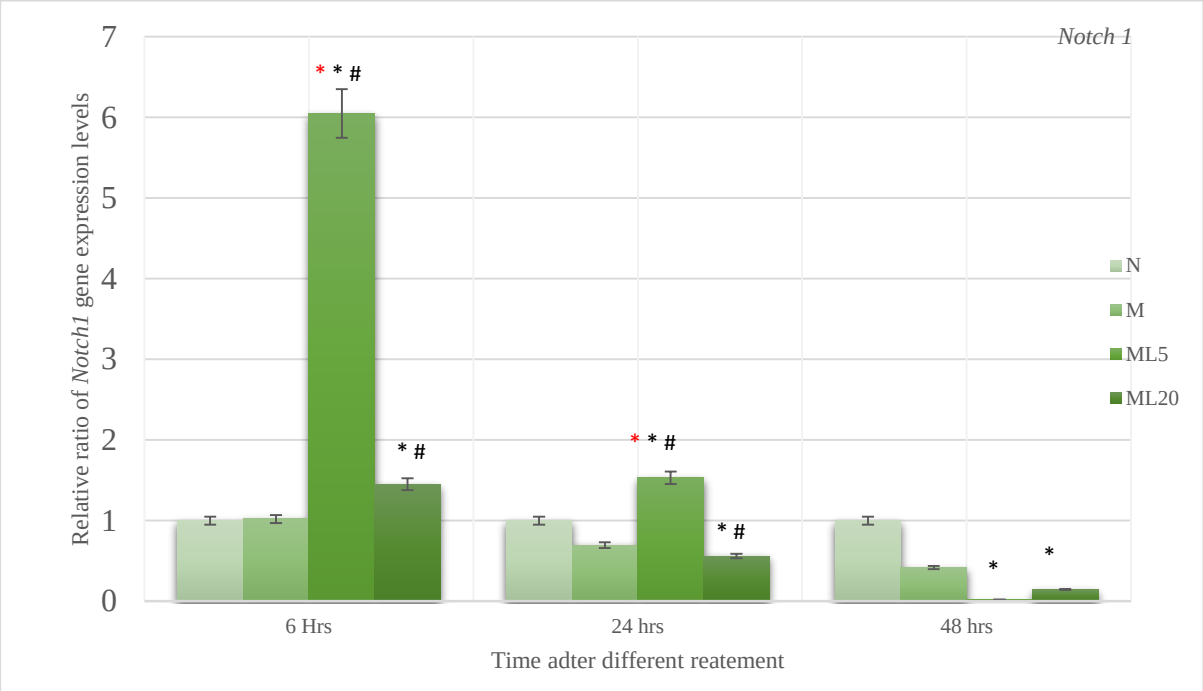
Figure 2 *JAG1 and JAG2* relative ratios' transcriptions level sin different treated and untreated ALL-cell lines' groups. *Where N Group represents untreated ALL-cell line as the negative control, M group represented ALL-cells treated with Methotrexate drug. ML5 and ML20 groups represent ALL cells treated with Methotrexate drug and 5 ug and 20 ug of Lactobacillus supernatant. A comparison was performed using the One-factor ANOVA test to analyse variance. (*) Significant at P<0.05. (*) Comparison between untreated control and treated groups. (#)Comparison between the M and the two concentrations of postbiotics probiotic treated groups. (*) Comparison between the three probiotic treated groups.*

Effects of the *L. acidophilus* free cell supernatant on the cell cycle controller gene transcriptions

In reckoning to *Notch*es receptors' transcription levels, by hour 6, the *Notch1* transcription level increased significantly in the ML5 compared to the ML20 group (P=0.0000). However, both ML5 and ML20 groups showed a significant increase when compared with their level in the untreated and M groups (P = 0.0000 and0.0000) (P = 0.0087 and 0.0114), respectively; Figure 3). By hour 24, the *Notch1* expression level changed to a significant downregulation in the ML20 group than those belonging to the untreated and ML5 cells groups (P = 0.0002 and 0.0001, respectively). In contrast, no conspicuous variances in the *Notch1* expression levels of the ML20 and M groups (Figure 3). Also, the *Notch 1* transcription level in the ML5 group still elevated significantly compared to its

corresponding levels in the untreated control and M groups after 24 hours (P= 0.0000, for each group, Figure 3). Ultimately, by hour 48, neither ML5 nor ML20 cells groups showed any significant differences in the *Notch 1* expression compared to the M group or in comparing to each other, but both groups showed a significant decrease in the *Notch1* level compared to those of the untreated. In reckoning to *Notch*es receptors' transcription levels, by hour 6, the expression level of the *Notch1* increased significantly in the ML5 compared to the ML20 group (P=0.0000). However, both ML5 and ML20 groups showed a significant increase when compared with their level in the untreated and M groups (P = 0.0000 and0.0000) (P = 0.0087 and 0.0114), respectively; Figure 3). By hour 24, the *Notch1* expression level changed to a significant downregulation in the ML20 group compared to those of the untreated control and ML5 groups (P = 0.0002 and 0.0001, respectively). In contrast, no conspicuous variances in the *Notch1* expression levels of the ML20 and M groups (Figure 3). Also, the *Notch 1* transcription level in the ML5 group still elevated significantly compared to its corresponding levels in the untreated control and M groups after 24 hours (P= 0.0000, for each group, Figure 3). Ultimately, by hour 48, neither ML5 nor ML20 cells groups showed any significant differences in the *Notch 1* expression compared to the M group or in comparing to each other, but both ML5 and ML20 treated cells showed a substantial decrease in the *Notch1* level compared to those of the untreated cells. Certainly, the current groups treated with *L. acidophilus* combined with Methotrexate chemotherapy showed an earlier intensify in the expression level of the *Notch1* control group (P= 0.0048; 0.0114, respectively; Figure 3). Certainly, the current groups treated with *L. acidophilus* combined with Methotrexate chemotherapy showed an earlier intensify in the transcription level of the *Notch1* control group (P= 0.0048; 0.0114, respectively; Figure 3).

By 6 hours from starting treatment, *Notch2* transcription levels diminished significantly in the ML5-cells group compared to its corresponding levels in the cell of N, M, and ML20 groups (P= 0.0012, 0.0000, and 0.0000, respectively). Meanwhile, the ML20-cells group showed a significant upregulation in the *Notch2* expression level compared to the N group (P=0.0001). In contrast, no conspicuous differences were observed in its expression level compared to the M group (Figure 3). After 24 hours from starting treatment, *Notch2* expression level still elevated with an extreme pattern in the ML20 cells compared to the N, M, and ML5 groups (P= 0.0000, for each group, respectively). *Notch2* transcription level in the ML5 cells group showed non-significant differences compared to the M cells group, though its level decreased significantly compared to the untreated cells group (P= 0.047; Figure 3). After 48 hours, the *Notch2* transcription level increased in a highly significant pattern in ML20-cells groups compared to the N, M, and ML5 groups (P= 0.0204, 0.0157, and 0.0046, respectively). Also, ALL-cells belonging to the ML5 group demonstrated non-significant differences in the transcription of *Notch2* levels than its corresponding levels in the N and M groups (Figure 3).



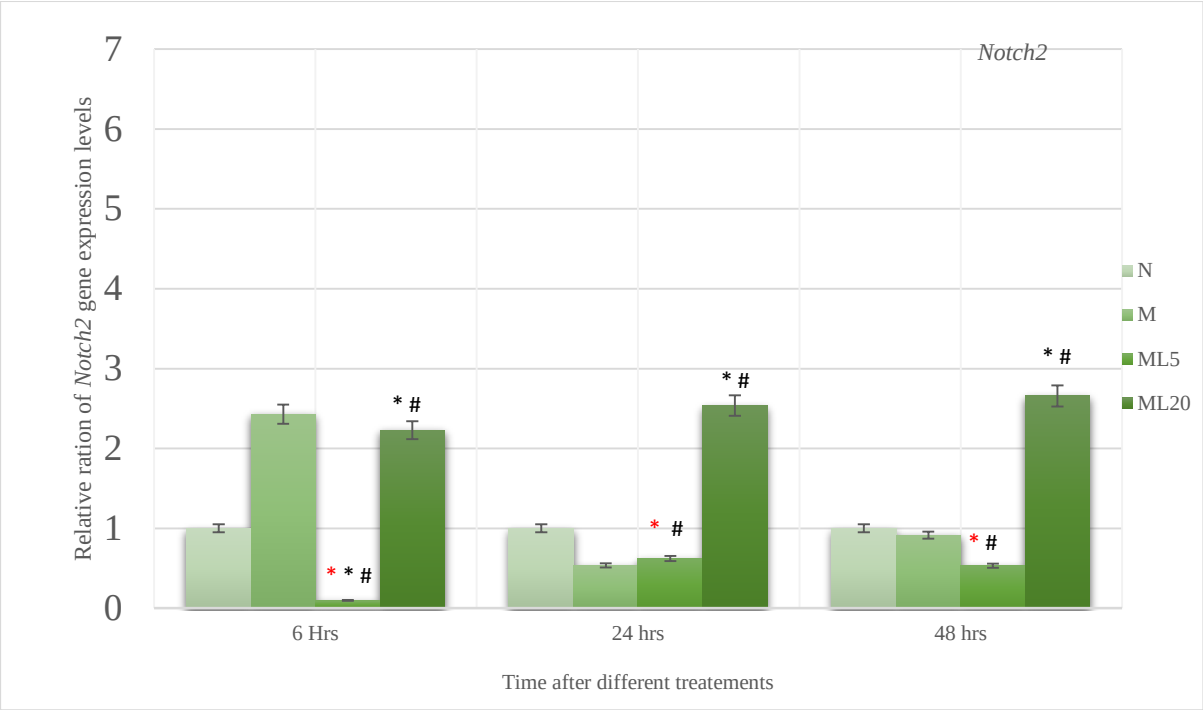


Figure 3 *Notch1, and Notch2,* relative ratios profiles transcriptions level sin different treated and untreated ALL-cell lines' groups. *Where N Group represents untreated ALL-cell line as the negative control, M group represented ALL-cells treated with Methotrexate drug. ML5 and ML20 groups represent ALL cells treated with Methotrexate drug and 5 ug and 20 ug of Lactobacillus supernatant. A comparison was performed using the One-factor ANOVA test to analyse variance. (*) Significant at P<0.05. (*) Comparison between untreated control and treated groups. (#) Comparison between the M and the two concentrations of postbiotics probiotic treated groups. (*) Comparison between the three probiotic treated groups.*

4. DISCUSSION

ALL is cancer affecting the bone marrow and the lymphatic system, which drive the bone marrow to produce nonfunctional white blood cells (Inaba and Mullighan, 2020). ALL is the most predominant childhood malignancy, where its prevalence was 30% of all cancers diagnosed in children under 15 years of age (Bahoush et al., 2021). However, almost 85% of ALL cases began from B lymphocytes, 15% began from T lymphocytes (Brunning, 2003). Methotrexate is the most frequent treatments against different cancers diseases (Dawson *et al*., 2004) and various autoimmune diseases during the past half-century (Fotoohi and Albertioni, 2008). The patient immune functioning is undoubtedly deactivated or defeated either due to the aggressive increase of the leukemia cells or even through the chemotherapy treatment (Vago and Gojo, 2020). Recently, some researchers provided various evidence of the probiotics -in particular, *Lactobacillus* and their products- as potential antitumor promoters (Greig, 2015), despite the notable proinflammatory effects associated with their dose increasing (El Hadad *et al*., 2019).

The critical factors of carcinogenesis depend upon stimulating apoptotic transcription genes, either pro or anti-apoptotic genes (Pistritto *et al*., 2016). In the current 6 hours from treatment, an earlier significant elevation was noticed in the *BAX* transcription levels in the ALL cells treated with Methotrexate and 0.5ug/ml of *L. acidophilus* supernatant free cells when compared with their corresponding levels in untreated M and ML20 cells groups. The BAX transcription levels still increased significantly by hour 24 from the treatment in the ML5 and ML20 cells than those belonging to the untreated cells, but it showed insignificant differences compared to their corresponding levels in the M cells. This significant elevation in the *BAX* transcription levels started diminishing by hour 48 from the treatment in the ML5 group compared with their corresponding levels in the N and M groups, but it diminished significantly compared to their corresponding levels in the ML20 group.

BAX transcription level reported a significant increase by hour 24 in the ALL cells treated with Methotrexate and 2ug/ml of *L. acidophilus* supernatant free cells compared to their corresponding levels in untreated. By hour 48, the *BAX* transcription level increased in an extremely significant manner in the ML20-ALL cells compared to its corresponding levels in untreated, Methotrexate alone, and Methotrexate combined with 0.5ug/ml of *L. acidophilus* supernatant free cells groups. The downregulation of the *BAX* expression is predominantly verified in various cancers (Ramadan *et al*., 2019). So, the current low and high concentrations of *L. acidophilus* supernatant associated with the methotrexate treatment stimulated the *BAX* expression, which may facilitate and encourage the apoptosis of ALL cells. Notably, the *BAX* gene is not only involved in many cellular activities but also is the most significant apoptotic activators (Ola *et al*., 2011).

NF-κB signaling presents a critical regulative role in the immune responses, either innate or adaptive. Its alteration has been confirmed in many immune deficiencies and cancerous diseases (Hoesel and Schmid, 2013). Several immune signals affect the activation of the *NF-κB* transcription, such as exposure to antigens, most of the Toll-like receptors, and proinflammatory cytokines (Gerondakis *et al*., 2014). A significant upregulation of the *NF-κB* transcription has been noticed in the present ML20-ALL cells starting from 24 hours and continuing till 48 hours compared with its corresponding levels in the untreated control, M, ML5 groups. However, the current ML5-ALL cells failed to increase the expression level of this tumor suppressor till 48 hours. This result signifies that the association of 2 ug/ml of *L. acidophilus* supernatant cell-free with Methotrexate chemotherapy enhances the *NF-κB* transcription level in the ALL cells, whereas *NF-κB* proteins are the central player in host immune responses activation against different antigens (Sun *et al*., 2013). The value of NF-κB transcription activation refers to its strength to provoke the transcription of pro-inflammatory genes (Sun, 2011), as IL-1 or TNF-α in the innate immune cells (Vallabhapurapu and Karin, 2009). The *NF-κB* transcription downregulation may cause severe immunodeficient, abnormal mitogen responses, and antibody generation deficiency (Zhang and Sun, 2015).

The *NF-κB* transcription level interferes with the *JAG1* transcription level in most cells, particularly cancerous cells (Zavadil *et al*., 2004). Despite its active role in *Notch* signaling in general (Whiteman et al., 2013), *JAG1* is an effective player in numerous aspects of cancer biology (Vizio *et al*., 2012). The current ALL-cells belonging to the ML5 group showed an early (after 6 hours) significant decrease in the *JAG1* transcription level compared to those of the untreated control, where this corresponding gene transcription level turned to nonsignificant differences after 24 and 48. Meanwhile, the *JAG1* transcription level fluctuated significantly in the ML5 cell group than those groups treated with Methotrexate alone after 6 and 24 hours. However, it increased non-significantly after 48 hours.

The present ALL-cells belonging to the ML20 showed a significant upregulation in the *JAG1* transcription level compared to untreated control at all the experiment periods, but this proapoptotic gene inhibited significantly after 6 hours then converted into a significant elevation compared to the groups treated with Methotrexate alone at hour 24 and 48. Several cancers feature such as neoplasm angiogenesis, neoplastic cell growth, and the metastatic process are affected directly by the transcription level of *JAG1* (Li *et al*., 2013). Also, *JAG1* plays a fundamental accomplishment in treatment resistance in many varieties of cancer (Liu *et al*., 2020). This implies the positive impacts of the *L. acidophilus* free cells supernatant on inhibiting this proapoptotic transcription level regardless of the supernatant concentrations compared to the group treated with MTX chemotherapy alone. Notably, many immunological parameters that are important in cancer -in particular the proinflammatory cytokines- such as TGF-β, and IL-6 stimulate the *JAG1* signaling pathway (Hong *et al*., 2010). The *JAG2* overexpression and cancer development correlations were previously noted in patients suffering from multiple myeloma (Houde et al., 2004). The *JAG2* gene not only upregulated significantly in various cancerous cells but also directly correlated to these neoplasms' progression and metastasis (Vizio *et al*., 2012).

In the current 6 hours, JAG2 transcription levels were decreased significantly in either ML5 or ML20 -cells groups than its corresponding level on MTX-cells group. These current results illustrated the early effective power of the *L. acidophilus'* supernatant, which inhibits the transcription level of the *JAG2* gene -defined as an effective tumorigenesis agent- in the ALL cells (Li *et al*., 2013). This downregulation in the current *JAG2* transcriptions has renewed after 24 and 48 hours and reached to a significant upregulation in the ALL-cells belonging to ML5 groups compared to untreated control and M groups. Meanwhile, its transcription level decreased in the ML20 group. Interestingly, colon cancer increased the *JAG2* expression level than those of the surrounding normal tissues, implying that differences in *JAG2* expression play a part in colon cancer development and progression towards the metastasis stages (Hong *et al*., 2010, Gaedcke *et al*., 2010). Moreover, these current results may propose an association between the concentration of the *Lactobacillus* supernatant needed for combination with the methotrexate chemotherapy and the expression level of *JAG2* levels. Remarkably, the well-defined action of *JAG2* in some types of cancers is still unclear, unlike the function of other *NOTCH* ligands such as *JAG1* in CRC that has been confirmed (Kim *et al*., 2013).

Notably, the *Notch* receptors are mammalian-specific receptors (Tan-Pertel *et al*., 2000) participating in the cells' progression (Richter *et al*., 2017). Generally, *Notch* signaling is perceived as an equilibrium keeper between cell proliferation, differentiation, and apoptosis (Miele *et al*., 2006), so they impersonate oncogenic or tumor suppressor roles depending on tissue context (Leong and Gao, 2008) several kinds of research confirmed the correlation between *Notch* gene dysregulation and many human malignancies (Graziani et al., 2008). The current *NOTCH* 1 expression level showed an extremely significant elevation in the ML5-ALL cells compared to its corresponding level in the untreated control, M, and ML20 groups after 6 and 24 hours, increasing its level diminished after 48 hours from starting treatments. This target gene expression level increased significantly in the ML20-ALL cells group than those belonging to the untreated and M groups after 6 hours, but this increase was declined at hour 24 till hour 48. In agreement with our results, *Notch* dysregulation has been noticed in hematopoiesis disorders (Hamidi *et al*., 2011), especially

Lymphoblastic leukemia (Katoh and Katoh, 2020). Remarkably, the lymphoid-related functions associated with *Notch* signaling were mentioned previously (Perchet *et al*., 2018).

The *Notch* signaling not only controls cell proliferation (Xin *et al*., 2010) , but also it established the differentiation of lymphocytes cells (Vinson *et al*., 2016). Also, *Notch* signaling is engaged in the apoptosis and cell death programming process through cell activation (Eagar *et al*., 2004), regulatory T cell function (Chen *et al*., 2019), and T helper cell differentiation (Amsen *et al*., 2007). Certainly, the current groups treated with *L. acidophilus* combined with Methotrexate chemotherapy showed an earlier increase in the expression level of the *NOTCH* gene announcing the readiness of the ALL cells for the apoptosis process (Eagar *et al*., 2004) earlier than those cells treated with Methotrexate alone. *Notch1* and *Notch2* are the most homologies with each other, whereas both in the extracellular and the intracellular domains' structures, nevertheless, they may have similar or opposite effects on the same tissues or disease (Miele *et al*., 2006).

The current six hours from treatment, *Notch2* expression level downregulated significantly in the ML5 group compared to its corresponding levels in the untreated, M and ML20 groups, though the ML20 group showed significant elevation when compared to the untreated cells and nonsignificant differences compared to the M group. Moreover, after 24 and 48 hours, the *Notch 2* expression level raised significantly in the ML20 cells group compared to the untreated M and ML5 group; meanwhile, the ML5 cells group showed nonsignificant differences in this target gene expression level compared to other groups

5. CONCLUSION

The present investigation verified the role of *L. acidophilus* cell-free supernatant on the transcription of the apoptotic genes of the ALL cell line during treatment with the Methotrexate chemotherapy. The current concentrations of *L. acidophilus* supernatant enhanced some of the pro-apoptotic genes like the initial activation in the transcription levels of *BAX*, *NF-KB*, *Notch1*, and *Notch2*. Nevertheless, this upregulation in the expression levels improved significantly after 48 hours from starting treatments and became extremely significant by increasing the concentration of the probiotic postbiotics. Remarkably, the down regulations of JAG1 and JAG2 (anti-apoptotic genes) expression levels in groups of ALL-cells treated with the *L. acidophilus* postbiotics extract and Methotrexate drug is evidence confirming the antitumor impacts of this postbiotic. Finally, the consequences of our study assure future research into different concentrations of other probiotic bacteria to determine their influences on apoptotic genes regulations during the treatment of different types of chemotherapy or even during radiotherapy.

Acknowledgments

ALL the authors would like to acknowledge King Fahad Center for Medical Research for aiding in the conduction of this study.

Author Contributions

Sahar EL Hadad designed the study, performed the experimental analyses, wrote, revised, and edited the manuscript. Hind Baik Financial support performed the experiments and participated in manuscript editing, Alawiah Alhebshi, Majdah Aburas, Tissue culture maintenance, and manuscript reviewing; Jehan Alrahimi and Shahira Hassoubah participated in gene expression testing and manuscript reviewing.

Ethical approval

The ethical approval cleared by the ethics committee of King Fahed for Medical Research Center (ethic No. 681-19).

Funding

This study has not received any external funding.

Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Ambalam P, Raman M, Purama RK, Doble M. Probiotics, prebiotics and colorectal cancer prevention. Best Pract Res Clin Gastroenterol 2016; 30: 119-131 doi: 10.1016/j.bpg.2016.02.009.
2. Amsen D, Antov A, Jankovic D, Sher A, Radtke F, Souabni A, Busslinger M, McCright B, Gridley T, Flavell RA. Direct regulation of Gata3 expression determines the T helper differentiation potential of Notch. Immunity 2007; 27:89-99 doi:10.1016/j.immuni.2007.05.021.
3. Bahoush G, Rahmati Z, Soheilipour F. Association between body mass index at diagnosis and outcome of children with Acute Lymphoblastic Leukemia. Med Sci 2021;25(108):312-319
4. Bedoui Y, Guillot X, Sélambarom J, Guiraud P, Giry C, Jaffar-Bandjee MC, Ralandison S, Gasque P. Methotrexate an old drug with new tricks. Int J Mol Sci 2019; 20 doi: 10.3390/ijms20205023.
5. Brunning RD. Classification of acute leukemias. Semin Diagn Pathol 2003; 20: 142-153 doi: 10.1016/s0740-2570(03)00031-5.
6. Chen ELY, Thompson PK, Zúñiga-Pflücker JC. RBPJ-dependent notch signaling initiates the T cell program in a subset of thymus-seeding progenitors. Nat immunol 2019; 20: 1456-1468 doi:10.1038/s41590-019-0518-7.
7. Choi SS, Kim Y, Han KS, You S, Oh S, Kim SH. Effects of Lactobacillus strains on cancer cell proliferation and oxidative stress in vitro. Lett Appl Microbiol 2006; 42: 452-458 doi:10.1111/j.1472-765X.2006.01913.x.
8. Conway R, Carey JJ. Risk of liver disease in methotrexate treated patients. World J hepatol 2017; 9:1092-1100 doi:10.4254/wjh.v9.i26.1092.
9. Csordas K, Lautner-Csorba O, Semsei AF, Harnos A, Hegyi M, Erdelyi DJ, Eipel OT, Szalai C, Kovacs GT. Associations of novel genetic variations in the folate-related and ARID5B genes with the pharmacokinetics and toxicity of high-dose methotrexate in paediatric acute lymphoblastic leukaemia. Br J Haematol 2014; 166: 410-420 doi:10.1111/bjh.12886.
10. Cuevas-González PF, Liceaga AM, Aguilar-Toalá JE. Postbiotics and paraprobiotics: From concepts to applications. Food Res Int (Ottawa, Ont.) 2020; 136: 109502 doi:10.1016/j.foodres.2020.109502.
11. Dawson J, Clewes A, Hendry J. Pulmonary effects of low-dose methotrexate therapy. Clin Pulm Med 2004; 11: 307-317 doi:10.1097/01.cpm.0000140182.17091.ef.
12. de Beaumais TA, Jacqz-Aigrain E. Intracellular disposition of methotrexate in acute lymphoblastic leukemia in children. Curr Drug Metab 2012; 13, 822-834 doi: 10.2174/138920012800840400.
13. Den Boer ML, Harms DO, Pieters R, Kazemier KM, Gobel U, Körholz D, Graubner U, Haas RJ, Jorch N, Spaar HJ, Kaspers GJ, Kamps WA, Van der Does-Van den Berg A, Van Wering ER, Veerman AJ, Janka-Schaub GE. Patient stratification based on prednisolone-vincristine-asparaginase resistance profiles in children with acute lymphoblastic leukemia. J Clin Oncol 2003; 21: 3262-3268 doi:10.1200/jco.2003.11.031.
14. Ding C, Tang W, Fan X, Wu G. Intestinal microbiota: a novel perspective in colorectal cancer biotherapeutics. Onco Targets Ther 2018; 11: 4797-4810 doi:10.2147/ott.S170626.
15. Eagar TN, Tang Q, Wolfe M, He Y, Pear WS, Bluestone JA. Notch 1 signaling regulates peripheral T cell activation. Immunity 2004; 20: 407-415 doi:10.1016/s1074-7613(04)00081-0.
16. El Hadad S, Zakareya A, Al-Hejin A, Aldahlawi A, Alharbi M. Sustaining exposure to high concentrations of bifidobacteria inhibits gene expression of Mouse's mucosal immunity. Heliyon 2019; 5: e02866 doi: 10.1016/j.heliyon.2019.e02866.
17. Fotoohi AK, Albertioni F. Mechanisms of antifolate resistance and methotrexate efficacy in leukemia cells. Leuk Lymphom 2008; 49: 410-426 doi: 10.1080/10428190701824569.
18. Gaedcke J, Grade M, Jung K, Camps J, Jo P, Emons G, Gehoff A, Sax U, Schirmer M, Becker H, Beissbarth T, Ried T, Ghadimi BM. Mutated KRAS results in overexpression of DUSP4, a MAP-kinase phosphatase, and SMYD3, a histone methyltransferase, in rectal carcinomas. Genes Chromosomes Cancer 2010; 49(11):1024-1034 doi: 10.1002/gcc.20811. PMID: 20725992; PMCID: PMC3535184.
19. Gerondakis S, Fulford TS, Messina NL, Grumont RJ. NF-κB control of T cell development. Nat immunol 2014; 15: 15-25 doi:10.1038/ni.2785.
20. Graux C. Biology of acute lymphoblastic leukemia (ALL): clinical and therapeutic relevance. Transfus Apher Sci 2011; 44(2):183-9 doi: 10.1016/j.transci.2011.01.009. Epub 2011 Feb 25. PMID: 21354375.
21. Graziani I, Eliasz S, De Marco MA, Chen Y, Pass HI, De May RM, Strack PR, Miele L, Bocchetta M. Opposite effects of Notch-1 and Notch-2 on mesothelioma cell survival under hypoxia are exerted through the Akt pathway. Cancer Res 2008; 68: 9678-9685 doi:10.1158/0008-5472.Can-08-0969.
22. Greig SL. Brexpiprazole: First Global Approval. Drugs 2015; 75: 1687-1697 doi:10.1007/s40265-015-0462-2.
23. Hadad SE, Hazmi BA, Alhebshi A, Aldahlawi AM, Bassam RA. Lactobacillus rhamnosus enhances the immunological antitumor effect of 5-fluorouracil against colon cancer. Pak J Biol Sci 2019; 22:597-606 doi:10.3923/pjbs.2019.597.606.
24. HadadSE, Alsolami M, Aldahlawi A, Alrahimi J, Basingab F, HassoubahS, Alothaid H. In vivo evidence: Repression of

mucosal immune responses in mice with colon cancer following sustained administration of Streptococcus thermophiles. Saudi J Biol Sci 2021; 28: 4751-4761 doi:10.1016/j.sjbs.2021.04.090.

25. Hamidi H, Gustafason D, Pellegrini M, Gasson J. Identification of novel targets of CSL dependent Notch signaling in hematopoiesis. PloS one 2011; 6: e20022 doi:10.1371/journal.pone.0020022.
26. Hansen MRW, Clausen A, Ejsing CS, Risbo J. Modulation of the Lactobacillus acidophilus La-5 lipidome by different growth conditions. Microbiol (Reading, England) 2015; 161: 1990-1998 doi:10.1099/mic.0.000145.
27. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014; 11(8):506-14 doi: 10.1038/nrgastro.2014.66. Epub 2014 Jun 10. PMID: 24912386.
28. Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. Mol Cancer 2013; 12: 86 doi:10.1186/1476-4598-12-86.
29. Hong Y, Downey T, Eu KW, Koh, PK, Cheah PY. A metastasis-prone' signature for early-stage mismatch-repair proficient sporadic colorectal cancer patients and its implications for possible therapeutics. Clin Exp Metastasis 2010; 27: 83-90 doi:10.1007/s10585-010-9305-4.
30. Houde C, Li Y, Song L, Barton K, Zhang Q, Godwin J, Nand S, Toor A, Alkan S, Smadja NV, Avet-Loiseau H, Lima CS, Miele L, Coignet LJ. Overexpression of the NOTCH ligand JAG2 in malignant plasma cells from multiple myeloma patients and cell lines. Blood 2004; 104(12):3697-704 doi: 10.1182/blood-2003-12-4114. Epub 2004 Aug 3. PMID: 15292061.
31. Hu YH, Zhou L, Wang SS, Jing X, Guo HL, Sun F, Zhang Y, Chen F, Xu J, Ji X. Methotrexate Disposition in Pediatric Patients with Acute Lymphoblastic Leukemia: What Have We Learnt From the Genetic Variants of Drug Transporters. Curr Pharm Des 2019; 25:627-634 doi:10.2174/1381612825666190329141003.
32. Huntly BJ, Gilliland DG. Leukaemia stem cells and the evolution of cancer-stem-cell research. Nature reviews. Cancer 2005; 5:311-321 doi:10.1038/nrc1592.
33. Inaba H, Mullighan CG. Pediatric acute lymphoblastic leukemia. Haematologica 2020; 105: 2524-2539 doi:10.3324/haematol.2020.247031.
34. Karimi Ardestani S, Tafvizi F, Tajabadi EM. Heat-killed probiotic bacteria induce apoptosis of HT-29 human colon adenocarcinoma cell line via the regulation of Bax/Bcl2 and caspases pathway. Hum Exp Toxicol 2019; 38: 1069-1081 doi:10.1177/0960327119851255.
35. Katoh M, Katoh M. Precision medicine for human cancers with Notch signaling dysregulation (Review). Int J Mol Med 2020; 45: 279-297 doi:10.3892/ijmm.2019.4418.
36. Kim MH, Kim HB, Yoon SP, Lim SC, Cha MJ, Jeon YJ, Park SG, Chang IY, You HJ. Colon cancer progression is driven by APEX1-mediated upregulation of Jagged. J Clin Investig 2013; 123: 3211-3230 doi:10.1172/jci65521.
37. Kim R. Recent advances in understanding the cell death pathways activated by anticancer therapy. Cancer 2005; 103: 1551-1560 doi:10.1002/cncr.20947.
38. Leong KG, Gao WQ. The Notch pathway in prostate development and cancer. Differentiation 2008; 76: 699-716 doi:10.1111/j.1432-0436.2008.00288.x.
39. Li W, Liu M, Feng Y, Huang YF, Xu YF, Che JP, Wang GC, Zheng JH. High expression of Notch ligand Jagged2 is associated with the metastasis and recurrence in urothelial carcinoma of bladder. Int J Clin Exp Path 2013; 6: 2430-2440.
40. Liu Z, Zhu Y, Li F, Xie Y. GATA1-regulated JAG1 promotes ovarian cancer progression by activating Notch signal pathway. Protoplasma 2020; 257: 901-910 doi:10.1007/s00709-019-01477-w.
41. Miele L, Miao H, Nickoloff BJ. NOTCH signaling as a novel cancer therapeutic target. Cur Cancer Drug Targets 2006; 6:313-323 doi:10.2174/156800906777441771.
42. Ola MS, Nawaz M, Ahsan H. Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. Mol Cell Biochem 2011; 351: 41-58 doi:10.1007/s11010-010-0709-x.
43. Perchet T, Petit M, Banchi EG, Meunier S, Cumano A, Golub R. The Notch Signaling Pathway Is Balancing Type 1 Innate Lymphoid Cell Immune Functions. Front Immunol 2018; 9: 1252 doi:10.3389/fimmu.2018.01252.
44. Pistritto G, Trisciuoglio D, Ceci C, Garufi A, D'Orazi G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging 2016; 8: 603-619 doi:10.18632/aging.100934.
45. Ramadan MA, Shawkey AE, Rabeh MA, Abdellatif AO. Expression of P53, BAX, and BCL-2 in human malignant melanoma and squamous cell carcinoma cells after tea tree oil treatment in vitro. Cytotechnol 2019; 71, 461-473 doi:10.1007/s10616-018-0287-4.
46. Richter J, Traver D, Willert K. The role of Wnt signaling in hematopoietic stem cell development. Crit Rev Biochem Mol Biol 2017; 52, 414-424 doi:10.1080/10409238.2017.1325828.
47. Saudi Cancer Registry MoH, Kingdom of Saudi Arabia (2013). Cancer Incidence and Survival Report; Saudi Arabia. Accessed (01-06-2016).
48. Strober W. Trypan Blue Exclusion Test of Cell Viability. Curr Protoc Immunol 2015; 111: A3.B.1-a3.B.3 doi: 10.1002/0471142735.ima03bs111.

49. Sun SC, Chang JH, Jin J. Regulation of nuclear factor-κB in autoimmunity. Trends Immunol 2013; 34: 282-289 doi:10.1016/j.it.2013.01.004.
50. Sun SC. Non-canonical NF-κB signaling pathway. Cell Res 2011; 21: 71-85 doi:10.1038/cr.2010.177.
51. Tan-Pertel HT, Walker L, Browning D, Miyamoto A, Weinmaster G, Gasson JC. Notch signaling enhances survival and alters differentiation of 32D myeloblasts. J Immunol 2000; 165: 4428-4436 doi: 10.4049/jimmunol.165.8.4428.
52. Vago L, Gojo I. Immune escape and immunotherapy of acute myeloid leukemia. J Clin Investig 2020; 130: 1552-1564 doi:10.1172/jci129204.
53. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. Ann Rev Immunol 2009; 27: 693-733 doi: 10.1146/annurev.immunol.021908.132641.
54. Végran F, Boidot R, Bonnetain F, Cadouot M, Chevrier S, Lizard-Nacol S. Apoptosis gene signature of Survivin and its splice variant expression in breast carcinoma. Endocr.-Relat. Cancer 2011; 18: 783-792 doi:10.1530/erc-11-0105.
55. Vinson KE, George DC, Fender AW, Bertrand FE, Sigounas G. The Notch pathway in colorectal cancer. Intl J Cancer 2016; 138: 1835-1842 doi:10.1002/ijc.29800.
56. Vizio B, Mauri FA, Prati A, Trivedi P, Giacobino A, Novarino A, Satolli MA, Ciuffreda L, Camandona M, Gasparri, G, Bellone G. Comparative evaluation of cancer stem cell markers in normal pancreas and pancreatic ductal adenocarcinoma. Oncol Rep 2012; 27:69-76 doi: 10.3892/or.2011.1461.
57. Whiteman P, de Madrid BH, Taylor P, Li D, Heslop R, Viticheep N, Tan JZ, Shimizu H, Callaghan J, Masiero M, Li JL, Banham AH, Harris AL, Lea SM, Redfield C, Baron M, Handford PA. Molecular basis for Jagged-1/Serrate ligand recognition by the Notch receptor. J Biol Chem 2013; 288(10):7305-12 doi: 10.1074/jbc.M112.428854. PMID: 23339193; PMCID: PMC3591638.
58. Wiewiórowski M, Graczyk J. The effective of methotrexate on immune response cells in rheumatoid arthritis. Acta Pol Pharm 2000; 57 Suppl: 138-139.
59. Xin Y, Lu Q, Li Q. 14-3-3sigma controls corneal epithelial cell proliferation and differentiation through the Notch signaling pathway. Biochem Biophys Res Commun 2010; 392: 593-598 doi:10.1016/j.bbrc.2010.01.084.
60. Zavadil J, Cermak L, Soto-Nieves N, Böttinger EP. Integration of TGF-beta/Smad and Jagged1/Notch signalling in epithelial-to-mesenchymal transition. Embo J 2004; 23: 1155-1165 doi:10.1038/sj.emboj.7600069.
61. Zhang H, Sun SC. NF-κB in inflammation and renal diseases. Cell Biosci 2015; 5: 63 doi: 10.1186/s13578-015-0056-4.